

We claim:

1. A method for cleaving single-stranded nucleic acid sequences at a desired location, the method comprising the steps of:

- 5 (i) contacting the nucleic acid with a single-stranded oligonucleotide, the oligonucleotide being functionally complementary to the nucleic acid in the region in which cleavage is desired and including a sequence that with its complement in the nucleic acid forms a restriction endonuclease recognition site that on restriction results in cleavage of the nucleic acid at the desired location; and
- 10 (ii) cleaving the nucleic acid solely at the recognition site formed by the complementation of the nucleic acid and the oligonucleotide;
- 15

the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large enough region to allow the two strands to associate such that cleavage may occur at the chosen temperature and at the desired location, and the cleavage being carried out using a restriction endonuclease that is active at the chosen temperature.

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2. A method for cleaving single-stranded nucleic acid sequences at a desired location, the method comprising the steps of:

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- (i) contacting the nucleic acid with a partially double-stranded oligonucleotide,

the single-stranded region of the
oligonucleotide being functionally
complementary to the nucleic acid in the
region in which cleavage is desired, and the
5 double-stranded region of the oligonucleotide
having a restriction endonuclease recognition
site; and

(ii) cleaving the nucleic acid solely at
the restriction endonuclease recognition site
10 formed by the complementation of the nucleic
acid and the single-stranded region of the
oligonucleotide;

the contacting and the cleaving steps being performed
at a temperature sufficient to maintain the nucleic
15 acid in substantially single-stranded form, the
oligonucleotide being functionally complementary to the
nucleic acid over a large enough region to allow the
two strands to associate such that cleavage may occur
at the chosen temperature and at the desired location,
20 and the cleavage being carried out using a restriction
endonuclease that is active at the chosen temperature.

3. In a method for displaying a member of a
diverse family of peptides, polypeptides or proteins on
the surface of a genetic package and collectively
25 displaying at least a part of the diversity of the
family, the improvement being characterized in that the
displayed peptide, polypeptide or protein is encoded at
least in part by a nucleic acid that has been cleaved
at a desired location by a method comprising the steps
30 of:

(i) contacting the nucleic acid with a
single-stranded oligonucleotide, the

oligonucleotide being functionally
complementary to the nucleic acid in the
region in which cleavage is desired and
including a sequence that with its complement
5 in the nucleic acid forms a restriction
endonuclease recognition site that on
restriction results in cleavage of the
nucleic acid at the desired location; and
(ii) cleaving the nucleic acid solely at
10 the recognition site formed by the
complementation of the nucleic acid and the
oligonucleotide;

the contacting and the cleaving steps being performed
at a temperature sufficient to maintain the nucleic
15 acid in substantially single-stranded form, the
oligonucleotide being functionally complementary to the
nucleic acid over a large enough region to allow the
two strands to associate such that cleavage may occur
at the chosen temperature and at the desired location,
20 and the cleavage being carried out using a restriction
endonuclease that is active at the chosen temperature.

4. In a method for displaying a member of a
diverse family of peptides, polypeptides or proteins on
the surface of a genetic package and collectively
25 displaying at least a part of the diversity of the
family, the improvement being characterized in that the
displayed peptide, polypeptide or protein is encoded by
a DNA sequence comprising a nucleic acid that has been
cleaved at a desired location by
30 (i) contacting the nucleic acid with a
partially double-stranded oligonucleotide,
the single-stranded region of the

oligonucleotide being functionally
complementary to the nucleic acid in the
region in which cleavage is desired, and the
double-stranded region of the oligonucleotide
5 having a restriction endonuclease recognition
site; and

(ii) cleaving the nucleic acid solely at
the restriction endonuclease recognition
cleavage site formed by the complementation
10 of the nucleic acid and the single-stranded
region of the oligonucleotide;

the contacting and the cleaving steps being performed
at a temperature sufficient to maintain the nucleic
acid in substantially single-stranded form, the
15 oligonucleotide being functionally complementary to the
nucleic acid over a large enough region to allow the
two strands to associate such that cleavage may occur
at the chosen temperature and at the desired location,
and the cleavage being carried out using a restriction
20 endonuclease that is active at the chosen temperature.

5. A method for displaying a member of a
diverse family of peptides, polypeptides or proteins on
the surface of a genetic package and collectively
displaying at least a part of the diversity of the
25 family, the method comprising the steps of:

(i) preparing a collection of nucleic acids
that code at least in part for members of the diverse
family;

(ii) rendering the nucleic acids single-
30 stranded;

5 (a) contacting the nucleic acid with a
single-stranded oligonucleotide, the
oligonucleotide being functionally
complementary to the nucleic acid in the
region in which cleavage is desired and
including a sequence that with its complement
10 in the nucleic acid forms a restriction
endonuclease recognition site that on
restriction results in cleavage of the
nucleic acid at the desired location; and

the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large enough region to allow the two strands to associate such that cleavage may occur at the chosen temperature and at the desired location, and the cleavage being carried out using a restriction endonuclease that is active at the chosen temperature; and

(iv) displaying a member of the family of
30 peptides, polypeptides or proteins coded, at least in
part, by the cleaved nucleic acids on the surface of
the genetic package and collectively displaying at
least a portion of the diversity of the family.

6. A method for displaying a member of a diverse family of peptides, polypeptides or proteins on the surface of a genetic package and collectively displaying at least a portion of the diversity of the family, the method comprising the steps of:

(i) preparing a collection of nucleic acids that code, at least in part, for members of the diverse family;

(ii) rendering the nucleic acids single-stranded;

(iii) cleaving the single-stranded nucleic acids at a desired location by a method comprising the steps of:

(a) contacting the nucleic acid with a partially double-stranded oligonucleotide, the single-stranded region of the oligonucleotide being functionally complementary to the nucleic acid in the region in which cleavage is desired, and the double-stranded region of the oligonucleotide having a restriction endonuclease recognition site; and

(b) cleaving the nucleic acid solely at the restriction endonuclease recognition cleavage site formed by the complementation of the nucleic acid and the single-stranded region of the oligonucleotide;

the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large enough region to allow the two strands to associate such that cleavage may occur at the

chosen temperature and at the desired location,
and the restriction being carried out using a
cleavage endonuclease that is active at the chosen
temperature; and

5 (iv) displaying a member of the family of
peptides, polypeptides or proteins coded, at least in
part, by the cleaved nucleic acids on the surface of
the genetic package and collectively displaying at
least a portion of the diversity of the family.

10 7. In a method for expressing a member of a
diverse family of peptides, polypeptides or proteins
and collectively expressing at least a part of the
diversity of the family, the improvement being
characterized in that the expressed peptide,
15 polypeptide or protein is encoded at least in part by a
nucleic acid that has been cleaved at a desired
location by a method comprising the steps of:

(i) contacting the nucleic acid with a
single-stranded oligonucleotide, the
20 oligonucleotide being functionally
complementary to the nucleic acid in the
region in which cleavage is desired and
including a sequence that with its complement
in the nucleic acid forms a restriction
25 endonuclease recognition site that on
restriction results in cleavage of the
nucleic acid at the desired location; and

(ii) cleaving the nucleic acid solely at
the recognition site formed by the
30 complementation of the nucleic acid and the
oligonucleotide;

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the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large enough region to allow the two strands to associate such that cleavage may occur at the chosen temperature and at the desired location, and the cleavage being carried out using a restriction endonuclease that is active at the chosen temperature.

8. In a method for expressing a member of a diverse family of peptides, polypeptides or proteins and collectively expressing at least a part of the diversity of the family, the improvement being characterized in that the expressed peptide, polypeptide or protein is encoded by a DNA sequence comprising a nucleic acid that has been cleaved at a desired location by

(i) contacting the nucleic acid with a partially double-stranded oligonucleotide, the single-stranded region of the oligonucleotide being functionally complementary to the nucleic acid in the region in which cleavage is desired, and the double-stranded region of the oligonucleotide having a restriction endonuclease recognition site; and

(ii) cleaving the nucleic acid solely at the restriction endonuclease recognition cleavage site formed by the complementation of the nucleic acid and the single-stranded region of the oligonucleotide;

the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the
5 nucleic acid over a large enough region to allow the two strands to associate such that cleavage may occur at the chosen temperature and at the desired location, and the cleavage being carried out using a restriction endonuclease that is active at the chosen temperature.

- 10 9. A method for expressing a member of a diverse family of peptides, polypeptides or proteins and collectively expressing at least a part of the diversity of the family, the method comprising the steps of:
- 15 (i) preparing a collection of nucleic acids that code at least in part for members of the diverse family;
- (ii) rendering the nucleic acids single-stranded;
- 20 (iii) cleaving the single-stranded nucleic acids at a desired location by a method comprising the steps of:
- (a) contacting the nucleic acid with a single-stranded oligonucleotide, the
25 oligonucleotide being functionally complementary to the nucleic acid in the region in which cleavage is desired and including a sequence that with its complement in the nucleic acid forms a restriction
30 endonuclease recognition site that on restriction results in cleavage of the nucleic acid at the desired location; and

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(b) cleaving the nucleic acid solely at the recognition site formed by the complementation of the nucleic acid and the oligonucleotide;

5 the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large
10 enough region to allow the two strands to associate such that cleavage may occur at the chosen temperature and at the desired location, and the cleavage being carried out using a restriction endonuclease that is active at the
15 chosen temperature; and

(iv) expressing a member of the family of peptides, polypeptides or proteins coded, at least in part, by the cleaved nucleic acids and collectively expressing at least a portion of the diversity of the
20 family.

10. A method for expressing a member of a diverse family of peptides, polypeptides or proteins and collectively expressing at least a portion of the diversity of the family, the method comprising the
25 steps of:

(i) preparing a collection of nucleic acids that code, at least in part, for members of the diverse family;

(ii) rendering the nucleic acids single-
30 stranded;

(iii) cleaving the single-stranded nucleic acids at a desired location by a method comprising the steps of:

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- 5 (a) contacting the nucleic acid with a partially double-stranded oligonucleotide, the single-stranded region of the oligonucleotide being functionally complementary to the nucleic acid in the region in which cleavage is desired, and the double-stranded region of the oligonucleotide having a restriction endonuclease recognition site; and
- 10 (b) cleaving the nucleic acid solely at the restriction endonuclease recognition cleavage site formed by the complementation of the nucleic acid and the single-stranded region of the oligonucleotide;
- 15 the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large
- 20 enough region to allow the two strands to associate such that cleavage may occur at the chosen temperature and at the desired location, and the restriction being carried out using a cleavage endonuclease that is active at the chosen
- 25 temperature; and
- (iv) expressing a member of the family of peptides, polypeptides or proteins coded, at least in part, by the cleaved nucleic acids and collectively expressing at least a portion of the diversity of the
- 30 family.

11. A library comprising a collection of genetic packages that display a member of a diverse family of peptides, polypeptides or proteins and

collectively display at least a portion of the diversity of the family, the library being produced using the methods of claims 3, 4, 5 or 6.

12. A library comprising a collection of
5 genetic packages that display a member of a diverse family of peptides, polypeptides or proteins and that collectively display at least a portion of the family, the displayed peptides, polypeptides or proteins being encoded by DNA sequences comprising at least in part
10 sequences produced by cleaving single-stranded nucleic acid sequences at a desired location by a method comprising the steps of:

(i) contacting the nucleic acid with a
single-stranded oligonucleotide, the
15 oligonucleotide being functionally complementary to the nucleic acid in the region in which cleavage is desired and including a sequence that with its complement in the nucleic acid forms a restriction
20 endonuclease recognition site that on restriction results in cleavage of the nucleic acid at the desired location; and

(ii) cleaving the nucleic acid solely at
the recognition site formed by the
25 complementation of the nucleic acid and the oligonucleotide;

the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the
30 oligonucleotide being functionally complementary to the nucleic acid over a large enough region to allow the two strands to associate such that cleavage may occur

at the chosen temperature and at the desired location,
and the cleavage being carried out using a restriction
endonuclease that is active at the chosen temperature.

13. A library comprising a collection of
5 genetic packages that display a member of a diverse
family of peptides, polypeptides or proteins and that
collectively display at least a portion of the
diversity of the family of the displayed peptides,
polypeptides or proteins being encoded by DNA sequences
10 comprising at least in part sequences produced by
cleaving single-stranded nucleic acid sequences at a
desired location by a method comprising the steps of:

(i) contacting the nucleic acid with a
partially double-stranded oligonucleotide,
15 the single-stranded region of the
oligonucleotide being functionally
complementary to the nucleic acid in the
region in which cleavage is desired, and the
double-stranded region of the oligonucleotide
20 having a restriction endonuclease recognition
site; and

(ii) cleaving the nucleic acid solely at
the restriction endonuclease recognition
cleavage site formed by the complementation
25 of the nucleic acid and the single-stranded
region of the oligonucleotide;

the contacting and the cleaving steps being performed
at a temperature sufficient to maintain the nucleic
acid in substantially single-stranded form, the
30 oligonucleotide being functionally complementary to the
nucleic acid over a large enough region to allow the
two strands to associate such that cleavage may occur
at the chosen temperature and at the desired location,

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and the cleavage being carried out using a restriction endonuclease that is active at the chosen temperature.

14. A library comprising a collection of members of a diverse family of peptides, polypeptides
5 or proteins and collectively comprising at least a portion of the diversity of the family, the library being produced using the methods of claims 7, 8, 9 or 10.

15. A library comprising a collection of
10 members of a diverse family of peptides, polypeptides or proteins and collectively comprising at least a portion of diversity of the family, the peptides, polypeptides or proteins being encoded by DNA sequences comprising at least in part sequences produced by
15 cleaving single-stranded nucleic acid sequences at a desired location by a method comprising the steps of:

(i) contacting the nucleic acid with a single-stranded oligonucleotide, the oligonucleotide being functionally
20 complementary to the nucleic acid in the region in which cleavage is desired and including a sequence that with its complement in the nucleic acid forms a restriction endonuclease recognition site that on
25 restriction results in cleavage of the nucleic acid at the desired location; and

(ii) cleaving the nucleic acid solely at the recognition site formed by the complementation of the nucleic acid and the
30 oligonucleotide;

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the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large enough region to allow the two strands to associate such that cleavage may occur at the chosen temperature and at the desired location, and the cleavage being carried out using a restriction endonuclease that is active at the chosen temperature.

10 16. A library comprising a collection of members of a diverse family of peptides, polypeptides or proteins and collectively comprising at least a portion of the diversity of the family, the peptides, polypeptides or proteins being encoded by DNA sequences
15 comprising at least in part sequences produced by cleaving single-stranded nucleic acid sequences at a desired location by a method comprising the steps of:

(i) contacting the nucleic acid with a partially double-stranded oligonucleotide, the single-stranded region of the oligonucleotide being functionally complementary to the nucleic acid in the region in which cleavage is desired, and the double-stranded region of the oligonucleotide having a restriction endonuclease recognition site; and

(ii) cleaving the nucleic acid solely at the restriction endonuclease recognition cleavage site formed by the complementation of the nucleic acid and the single-stranded region of the oligonucleotide;

the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic

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acid in substantially single-stranded form, the
oligonucleotide being functionally complementary to the
nucleic acid over a large enough region to allow the
two strands to associate such that cleavage may occur
5 at the chosen temperature and at the desired location,
and the cleavage being carried out using a restriction
endonuclease that is active at the chosen temperature.

17. A library of claims 11, 12 or 13 wherein
10 the genetic packages are selected from the group of
phage, phagemid or yeast.

18. A library of claims 17 wherein the
genetic packages are selected are phage or phagemid.

19. The methods or libraries according
15 claims 2, 4, 6, 8, 10, 13 or 16 wherein in the
restriction endonuclease recognition site is for a
Type II-S restriction endonuclease.

20. The methods or libraries according to
claims 1 to 19, wherein the nucleic acid is cDNA.

21. The methods or libraries according to
20 any one of claims 1 to 20, wherein the nucleic acids
encode at least a portion of an immunoglobulin.

22. The methods or libraries according to
claim 21, wherein the immunoglobulin comprises a Fab or
25 single chain Fv.

23. The methods or libraries according to
claim 21 or 22, wherein the immunoglobulin comprises at
least portion of a heavy chain.

7. 11. 13. 15. 17. 19. 21. 23. 25.

24. The method or libraries according to claim 23, wherein the heavy chain is IgM, IgG, IgA, IgE or IgD.

25. The methods or libraries according to claim 23 or 24, wherein at least a portion of the heavy chain is human.

26. The methods or libraries according to claim 21 or 22, wherein the immunoglobulin comprises at least a portion of FR1.

27. The methods or libraries according to claim 26, wherein at least a portion of the FR1 is human.

28. The methods or libraries according to claim 21 or 22, wherein the immunoglobulin comprises at least a portion of a light chain.

29. The methods or libraries according to claim 28, wherein at least a portion of the light chain is human.

30. The methods or libraries according to any one of claims 1 to 16, wherein the nucleic acid sequences are at least in part derived from patients suffering from at least one autoimmune disease and/or cancer.

31. The methods or libraries according to claim 30, wherein the autoimmune disease is selected from the group comprising lupus, erythematosus,

systemic sclerosis, rheumatoid arthritis, antiphospholipid syndrome or vasculitis.

32. The methods or libraries according to claim 30, wherein the nucleic acids are at least in part isolated from the group comprising peripheral blood cells, bone marrow cells spleen cells or lymph node cells.

33. The methods according to claim 5, 6, 9 or 10 further comprising at least one nucleic acid amplification step between one or more of steps (i) and (ii), steps (ii) and (iii) or between steps (iii) and (iv).

34. The method according to claim 33,
wherein amplification primers for the amplification
15 step are functionally complementary to a constant
region of the nucleic acids.

35. The method according to claim 34,
wherein the constant region is genetically constant in
the nucleic acids.

20 36. The method according to claim 35,
wherein the genetically constant region is a part of
the genome of immunoglobulin genes selected from the
group of IgM, IgG, IgA, IgE or IgD.

37. The method according to claim 34,
25 wherein the constant region is exogenous to the nucleic
acids.

38. The methods according to claim 33,
wherein the amplification step uses generACE™.

39. The methods or libraries according to any one of claims 1 to 16, wherein the chosen temperature is between 37°C and 75°C

40. The methods or libraries according to claim 39, wherein the chosen temperature is between 45°C and 75°C.

41. The methods or libraries according to claim 40, wherein the chosen temperature is between 50°C and 60°C.

42. The methods or libraries according to claim 41, wherein the chosen temperature is between 55°C and 60°C.

43. The methods or libraries according to claim 1, 3, 5, 7, 9, 12 or 15, wherein the length of the single-stranded oligonucleotide is between 17 and 30 bases.

44. The methods or libraries according to claim 43, wherein the length of the single-stranded oligonucleotide is between 18 and 24 bases.

45. The methods or libraries according to claim 1, 3, 5, 7, 9, 12 or 15, wherein the restriction endonuclease is selected from the group comprising *MaeIII*, *Tsp45I*, *HphI*, *BsaJI*, *AluI*, *BlpI*, *DdeI*, *BglII*, *MslI*, *BsiEI*, *EaeI*, *EagI*, *HaeIII*, *Bst4CI*, *HpyCH4III*, *HinfI*, *MlyI*, *PleI*, *MnlI*, *HpyCH4V*, *BsmAI*, *BpmI*, *XmnI*, or *SacI*.

46. The methods or libraries according to claim 45, wherein the restriction endonuclease is selected from the group comprising *Bst4CI*, *TaaI*, *HpyCH4III*, *BlpI*, *HpyCH4V* or *MslI*.

5 47. The methods or libraries according to claim 2, 4, 6, 8, 10, 13 or 16, wherein the length of the single-stranded region of the partially double-stranded oligonucleotide is between 14 and 22 bases.

10 48. The methods or libraries according to claim 47, wherein the length of the single-stranded region of the partially double-stranded oligonucleotide is between 14 and 17 bases.

15 49. The methods or libraries according to claim 47, wherein the length of the single-stranded region of the oligonucleotide is between 18 and 20 bases.

20 50. The methods or libraries according to claim 2, 4, 6, 8, 10, 13 or 16, wherein the length of the double-stranded region of the partially double-stranded oligonucleotide is between 10 and 14 base pairs formed by a stem and its palindrome.

25 51. The methods or libraries according to claim 50 wherein, the partially double-stranded oligonucleotide comprises a loop of 3 to 8 bases between the stem and the palindrome.

52. The methods or libraries according to claim 19 wherein the Type II-S restriction endonuclease

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is selected from the group comprising AarICAC, AceIII, Bbr7I, BbvI, BbvII, Bce83I, BceAI, BcefI, BciVI, BfiI, BinI, BscAI, BseRI, BsmFI, BspMI, EciI, Eco57I, FauI, FokI, GsuI, HgaI, HphI, MboII, MlyI, MmeI, MnlI, PleI, 5 RleAI, SfaNI, SspD5I, Sth132I, StsI, TaqII, Tth111III, or UbaPI.

53. The methods or libraries according to claim 52, wherein the Type II-S restriction endonuclease is *FokI*.

- 10 54. A method for preparing single-stranded nucleic acids, the method comprising the steps of:
- (i) contacting a single-stranded nucleic acid sequence that has been cleaved with a restriction endonuclease with a partially double-stranded oligonucleotide, the single-stranded region of the oligonucleotide being functionally complementary to the nucleic acids in the region that remains after cleavage, the double-stranded region of the oligonucleotide including any sequences necessary to return the sequences that remain after cleavage into proper and original reading frame for expression and containing a restriction endonuclease recognition site 5' of those sequences; and
- 25 (ii) cleaving the partially double-stranded oligonucleotide sequence solely at the restriction endonuclease recognition site contained within the double-stranded region of the partially double-stranded oligonucleotide.
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the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the
5 nucleic acid over a large enough region to allow the two strands to associate such that cleavage may occur at the chosen temperature and at the desired location, and the cleavage being carried out using a restriction endonuclease that is active at the chosen temperature.

10 55. The method according to claim 54, wherein the length of the single-stranded portion of the partially double-stranded oligonucleotide is between 2 and 15 bases.

15 56. The method according to claim 55, wherein the length of the single-stranded portion of the partially double-stranded oligonucleotide is between 7 and 10 bases.

20 57. The method according to claim 54, wherein the length of the double-stranded portion of the partially double-stranded oligonucleotide is between 12 and 100 base pairs.

25 58. The method according to claim 57, wherein the length of the double-stranded portion of the partially double-stranded oligonucleotide is between 20 and 100 base pairs.

59. A method for preparing a library comprising a collection of genetic packages that display a member of a diverse family of peptides,

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polypeptides or proteins and that collectively display at least a portion of the family comprising the steps:

(i) preparing a collection of nucleic acids that code at least in part for members of the diverse
5 family;

(ii) rendering the nucleic acids single-stranded;

(iii) cleaving the single-stranded nucleic acids at a desired location by a method comprising the
10 steps of:

(a) contacting the nucleic acid with a single-stranded oligonucleotide, the oligonucleotide being functionally complementary to the nucleic acid in the
15 region in which cleavage is desired and including a sequence that with its complement in the nucleic acid forms a restriction endonuclease recognition site that on restriction results in cleavage of the
20 nucleic acid at the desired location; and

(b) cleaving the nucleic acid solely at the recognition site formed by the complementation of the nucleic acid and the oligonucleotide;

25 the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large
30 enough region to allow the two strands to associate such that cleavage may occur at the chosen temperature and at the desired location, and the cleavage being carried out using a

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restriction endonuclease that is active at the
chosen temperature;

(iv) contacting the nucleic acid with a
partially double-stranded oligonucleotide, the single-
5 stranded region of the oligonucleotide being
functionally complementary to the nucleic acids in the
region that remains after the cleavage in step (iii)
has been effected, and the double-stranded region of
the oligonucleotide including any sequences necessary
10 to return the sequences that remain after cleavage into
proper and original reading frame for display and
containing a restriction endonuclease recognition site
5' of those sequences that is different from the
restriction site used in step (iii); and

15 (v) cleaving the nucleic acid solely at the
restriction endonuclease recognition cleavage site
contained within the double-stranded region of the
partially double-stranded oligonucleotide;

the contacting and the cleaving steps being
20 performed at a temperature sufficient to maintain
the nucleic acid in substantially single-stranded
form, the oligonucleotide being functionally
complementary to the nucleic acid over a large
enough region to allow the two strands to
25 associate such that cleavage may occur at the
chosen temperature and at the desired location,
and the restriction being carried out using a
cleavage endonuclease that is active at the chosen
temperature; and

30 (vi) displaying a member of the family of
peptides, polypeptides or proteins coded, at least in
part, by the cleaved nucleic acids on the surface of
the genetic package and collectively displaying at
least a portion of the diversity of the family.

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60. A method for preparing a library
comprising a collection of members of a diverse family
of peptides, polypeptides or proteins and collectively
comprising at least a portion of the family comprising
5 the steps:

(i) preparing a collection of nucleic acids
that code at least in part for members of the diverse
family;

(ii) rendering the nucleic acids single-
10 stranded;

(iii) cleaving the single-stranded nucleic
acids at a desired location by a method comprising the
steps of:

(a) contacting the nucleic acid with a
15 single-stranded oligonucleotide, the
oligonucleotide being functionally
complementary to the nucleic acid in the
region in which cleavage is desired and
including a sequence that with its complement
20 in the nucleic acid forms a restriction
endonuclease recognition site that on
restriction results in cleavage of the
nucleic acid at the desired location; and

(b) cleaving the nucleic acid solely at
25 the recognition site formed by the
complementation of the nucleic acid and the
oligonucleotide;

the contacting and the cleaving steps being
performed at a temperature sufficient to maintain
30 the nucleic acid in substantially single-stranded
form, the oligonucleotide being functionally
complementary to the nucleic acid over a large
enough region to allow the two strands to
associate such that cleavage may occur at the

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chosen temperature and at the desired location,
and the cleavage being carried out using a
restriction endonuclease that is active at the
chosen temperature;

5 (iv) contacting the nucleic acid with a
partially double-stranded oligonucleotide, the single-
stranded region of the oligonucleotide being
functionally complementary to the nucleic acids in the
region that remains after the cleavage in step (iii)
10 has been effected, and the double-stranded region of
the oligonucleotide including any sequence necessary to
return the sequences that remain after cleavage into
proper and original reading frame for expression and
containing a restriction endonuclease recognition site
15 5' of those sequences that is different from the
restriction site used in step (iii); and

(v) cleaving the nucleic acid solely at the
restriction endonuclease recognition cleavage site
contained within the double-stranded region of the
20 partially double-stranded oligonucleotide;
the contacting and the cleaving steps being
performed at a temperature sufficient to maintain
the nucleic acid in substantially single-stranded
form, the oligonucleotide being functionally
25 complementary to the nucleic acid over a large
enough region to allow the two strands to
associate such that cleavage may occur at the
chosen temperature and at the desired location,
and the restriction being carried out using a
30 cleavage endonuclease that is active at the chosen
temperature; and

(vi) expressing a member of the family of
peptides, polypeptides or proteins coded, at least in
part, by the cleaved nucleic acids and collectively

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expressing at least a portion of the diversity of the family.

61. The methods according to claim 59 or 60, further comprising at least one nucleic acid
5 amplification step between one or more of steps (i) and (ii), steps (ii) and (iii), steps (iii) and (iv) and steps (iv) and (v).

62. A library comprising a collection of genetic packages that display a member of a diverse
10 family of peptides, polypeptides or proteins and collectively display at least a portion of the diversity of the family, the library being produced using the methods of claims 59 or 61.

63. A library comprising a collection of
15 members of a diverse family of peptides, polypeptides or proteins and collectively comprise at least a portion of the diversity of the family, the library being produced using the methods of claims 60 or 61.

64. The methods and libraries according to
20 any one of claim 59 to 63, wherein the members of the library encode immunoglobulins.

65. The method and libraries according to claim 64, wherein the double-stranded region of the oligonucleotide encodes at least a part of a framework
25 sequence of an immunoglobulin.

66. The method and libraries according to claim 65, wherein the framework sequence comprises framework 1 of an antibody.

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67. The method and libraries according to claim 66, wherein the framework sequence comprises framework 1 of a variable domain of a light chain.

68. The method and libraries according to
5 claim 66, wherein the framework sequence comprises framework 1 of a variable domain of a heavy chain.

69. The method and libraries according to claim 65, wherein the framework sequence comprises framework 3 of an antibody.

10 70. The method and libraries according to claim 69, wherein the framework sequence comprises framework 3 of a variable domain of a light chain.

71. The method and libraries according to claim 69, wherein the framework sequence is framework 3
15 of a variable domain of a heavy chain.

72. The method and libraries according to claim 66, wherein the 5' primer is complementary to a region outside framework 1.

73. The method according to claim 61,
20 wherein amplification primers for the amplification step are functionally complementary to a constant region of the nucleic acids.

74. The method according to claim 73,
wherein the constant region is genetically constant in
25 the nucleic acids.

75. The method according to claim 74, wherein the genetically constant region is part of the genome of immunoglobulin genes selected from the group of IgM, IgG, IgA, IgE or IgD.

5 76. The method according to claim 73, wherein the constant region is exogenous to the nucleic acids.

77. The methods according to claim 61, wherein the amplification step uses geneRACE™.

10 78. A vector comprising:
 (i) a DNA sequence encoding an antibody variable region linked to a version of PIII anchor which does not mediate infection of phage particles; and
15 (ii) wild-type gene III.

79. The vector according to claim 78, wherein the DNA encodes a Fab.

80. The vector according to claim 78, wherein the DNA encodes heavy chain VHCH1.

20 81. The vector according to claim 80, wherein the heavy chain VHCH1 is linked to trpIII.

82. The vector according to claim 78, wherein the DNA encodes light chain VLCL.

25 83. The vector according to claim 82, wherein the light chain VLCL is linked to trpIII.

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84. The vector according to claim 78,
wherein the DNA encodes scFv.

85. The vector according to claim 84,
wherein the scFv is VL-VH.

5 86. The vector according to claim 84,
wherein the scFv is VH-VL.

87. The vector according to claim 78,
wherein the DNA sequence encoding an antibody variable
region linked to a version of PIII anchor further
10 comprises an inducible promoter.

88. The vector according to claim 87,
wherein the inducible promoter regulates expression of
the DNA sequence encoding an antibody variable region
linked to a version of PIII anchor.

15 89. The vector according to claim 78,
wherein the DNA sequence encoding an antibody variable
region linked to a version of PIII anchor further
comprises an amber stop codon.

90. The vector according to claim 89,
20 wherein the DNA encoding the amber stop codon is
located between the antibody variable region and the
version of pIII.

91. The vector according to any one of
claims 78 to 90 wherein the vector is phage or
25 phagemid.

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92. A method for producing a population of immunoglobulin genes that comprises steps of:

- 5 (i) introducing synthetic diversity into at least one of CDR1 or CDR2 of those genes; and
- (ii) combining the diversity from step (i) with CDR3 diversity captured from B cells.

93. The method according to claim 92,
10 wherein synthetic diversity is introduced into both CDR1 and CDR2.

94. A method for producing a library of immunoglobulin genes that comprises

- 15 (i) introducing synthetic diversity into at least one of CDR1 or CDR2 of those genes; and
- (ii) combining the diversity from step (i) with CDR3 diversity captured from B cells.

20 95. The method according to claim 94, wherein synthetic diversity is introduced into both CDR1 and CDR2.

96. A library of immunoglobulins that comprise members with at least one variable domain in
25 which at least one of CDR1 and CDR2 contain synthetic diversity and CDR3 diversity is captured from B cells.

97. A library according to claim 96, where both CDR1 and CDR2 contain synthetic diversity.

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98. The vector according to claim 78,
wherein the version of PIII anchor is characterized by
a wild type amino acid sequence and is encoded by a
non-wild type degenerate DNA sequence to a very high
5 extent.

99. In a method for displaying a member of a
diverse family of peptides, polypeptides or proteins on
the surface of a genetic package and collectively
displaying at least a part of the diversity of the
10 family, the improvement being characterized in that the
displayed peptide, polypeptide or protein is encoded by
a DNA sequence comprising a nucleic acid that has been
cleaved at a desired location by

(i) contacting the nucleic acid with a
15 partially double-stranded oligonucleotide,
the single-stranded region of the
oligonucleotide being functionally
complementary to the nucleic acid at its 5'
terminal and

(ii) cleaving the nucleic acid solely at
20 a restriction endonuclease cleavage site
located in the double-stranded region of the
oligonucleotide or amplifying the nucleic
acid using a primer at least in part
25 functionally complementary to at least a part
of the double-stranded region of the
oligonucleotide, the primer also introducing
on amplification an endonuclease cleavage
site and cleaving the amplified nucleic acid
30 sequence solely at that site;

the contacting and the cleaving steps being performed
at a temperature sufficient to maintain the nucleic

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acid in substantially single-stranded form, the
oligonucleotide being functionally complementary to the
nucleic acid over a large enough region to allow the
two strands to associate such that cleavage may occur
5 at the chosen temperature and at the desired location,
and the cleavage being carried out using a restriction
endonuclease that is active at the chosen temperature.

100. A method for displaying a member of a
diverse family of peptides, polypeptides or proteins on
10 the surface of a genetic package and collectively
displaying at least a portion of the diversity of the
family, the method comprising the steps of:

(i) preparing a collection of nucleic acids
that code, at least in part, for members of the diverse
15 family;

(ii) rendering the nucleic acids single-
stranded;

(iii) cleaving the single-stranded nucleic
acids at a desired location by a method comprising the
20 steps of:

(a) contacting the nucleic acid with a
partially double-stranded oligonucleotide,
the single-stranded region of the
oligonucleotide being functionally
25 complementary to the nucleic acid at its 5'
terminal region; and

(b) cleaving the nucleic acid solely at
a restriction endonuclease cleavage site
located in the double-stranded region of the
oligonucleotide or amplifying the nucleic
30 acid using a primer at least in part
functionally complementary to at least a part
of the double-stranded region of the

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oligonucleotide, the primer also introducing on amplification an endonuclease cleavage site and cleaving the amplified nucleic acid sequence solely at that site;

- 5 the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large
10 enough region to allow the two strands to associate such that cleavage may occur at the chosen temperature and at the desired location, and the restriction being carried out using a cleavage endonuclease that is active at the chosen
15 temperature; and
- (iv) displaying a member of the family of peptides, polypeptides or proteins coded, at least in part, by the cleaved nucleic acids on the surface of the genetic package and collectively displaying at
20 least a portion of the diversity of the family.

101. In a method for expressing a member of a diverse family of peptides, polypeptides or proteins and collectively expressing at least a part of the diversity of the family, the improvement being
25 characterized in that the expressed peptide, polypeptide or protein is encoded by a DNA sequence comprising a nucleic acid that has been cleaved at a desired location by

- (i) contacting the nucleic acid with a
30 partially double-stranded oligonucleotide, the single-stranded region of the oligonucleotide being functionally

complementary to the nucleic acid at its 5' terminal region; and

5 (ii) cleaving the nucleic acid solely at the restriction endonuclease cleavage site located in the double-stranded region of the oligonucleotide or amplifying the nucleic acid using a primer at least in part functionally complementary to at least a part of the double-stranded region of the
10 oligonucleotide, the primer also introducing on amplification an endonuclease cleavage site and cleaving the amplified nucleic acid sequence solely at that site;

the contacting and the cleaving steps being performed
15 at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large enough region to allow the two strands to associate such that cleavage may occur
20 at the chosen temperature and at the desired location, and the cleavage being carried out using a restriction endonuclease that is active at the chosen temperature.

102. A method for expressing a member of a diverse family of peptides, polypeptides or proteins
25 and collectively expressing at least a portion of the diversity of the family, the method comprising the steps of:

(i) preparing a collection of nucleic acids that code, at least in part, for members of the diverse
30 family;

(ii) rendering the nucleic acids single-stranded;

(iii) cleaving the single-stranded nucleic acids at a desired location by a method comprising the
5 steps of:

(a) contacting the nucleic acid with a partially double-stranded oligonucleotide, the single-stranded region of the oligonucleotide being functionally
10 complementary to the nucleic acid at its 5' terminal region; and

(b) cleaving the nucleic acid solely at a restriction endonuclease cleavage site located in the double-stranded region of the
15 nucleotide; or amplifying the nucleic acid using a primer at least in part functionally complementary to at least a part of the double-stranded region of the oligonucleotide, the primer also introducing
20 on amplification an endonuclease cleavage site and cleaving the amplified nucleic acid sequence solely at that site;

the contacting and the cleaving steps being performed at a temperature sufficient to maintain
25 the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large enough region to allow the two strands to associate such that cleavage may occur at the
30 chosen temperature and at the desired location, and the restriction being carried out using a cleavage endonuclease that is active at the chosen temperature; and

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(iv) expressing a member of the family of peptides, polypeptides or proteins coded, at least in part, by the cleaved nucleic acids and collectively expressing at least a portion of the diversity of the family.

103. A method for preparing a library comprising a collection of genetic packages that display a member of a diverse family of peptides, polypeptides or proteins and that collectively display at least a portion of the family comprising the steps:

(i) preparing a collection of nucleic acids that code at least in part for members of the diverse family;

(ii) rendering the nucleic acids single-stranded;

(iii) cleaving the single-stranded nucleic acids at a desired location by a method comprising the steps of:

(a) contacting the nucleic acid with a single-stranded oligonucleotide, the oligonucleotide being functionally complementary to the nucleic acid in the region in which cleavage is desired and including a sequence that with its complement in the nucleic acid forms a restriction endonuclease recognition site that on restriction results in cleavage of the nucleic acid at the desired location; and

(b) cleaving the nucleic acid solely at the recognition site formed by the complementation of the nucleic acid and the oligonucleotide;

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the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large enough region to allow the two strands to associate such that cleavage may occur at the chosen temperature and at the desired location, and the cleavage being carried out using a restriction endonuclease that is active at the chosen temperature;

(iv) contacting the nucleic acid with a partially double-stranded oligonucleotide, the single-stranded region of the oligonucleotide being functionally complementary to the nucleic acids in the 5' terminal region that remains after the cleavage in step (iii) has been effected, and the double-stranded region of the oligonucleotide including any sequences necessary to return the sequences that remain after cleavage into proper and original reading frame for display; and

(v) cleaving the nucleic acid solely at a restriction endonuclease cleavage site contained within the double-stranded region of the partially double-stranded oligonucleotide, the site being different from that used in step (iii) or amplifying the nucleic acid using a primer at least in part functionally complementary to at least a part of the double-stranded region of the oligonucleotide, the primer also introducing on amplification an endonuclease cleavage site and cleaving the amplified nucleic acid sequence solely at that site;

the contacting and the cleaving steps being performed at a temperature sufficient to maintain

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the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large enough region to allow the two strands to
5 associate such that cleavage may occur at the chosen temperature and at the desired location, and the restriction being carried out using a cleavage endonuclease that is active at the chosen temperature; and

10 (vi) displaying a member of the family of peptides, polypeptides or proteins coded, at least in part, by the cleaved nucleic acids on the surface of the genetic package and collectively displaying at least a portion of the diversity of the family.

15 104. A method for preparing a library comprising a collection of members of a diverse family of peptides, polypeptides or proteins and collectively comprising at least a portion of the family comprising the steps:

20 (i) preparing a collection of nucleic acids that code at least in part for members of the diverse family;

(ii) rendering the nucleic acids single-stranded;

25 (iii) cleaving the single-stranded nucleic acids at a desired location by a method comprising the steps of:

(a) contacting the nucleic acid with a single-stranded oligonucleotide, the
30 oligonucleotide being functionally complementary to the nucleic acid in the region in which cleavage is desired and including a sequence that with its complement

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in the nucleic acid forms a restriction endonuclease recognition site that on restriction results in cleavage of the nucleic acid at the desired location; and

5 (b) cleaving the nucleic acid solely at the recognition site formed by the complementation of the nucleic acid and the oligonucleotide;

10 the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large enough region to allow the two strands to
15 associate such that cleavage may occur at the chosen temperature and at the desired location, and the cleavage being carried out using a restriction endonuclease that is active at the chosen temperature;

20 (iv) contacting the nucleic acid with a partially double-stranded oligonucleotide, the single-stranded region of the oligonucleotide being functionally complementary to the nucleic acids in the 5' terminal region that remains after the cleavage in
25 step (iii) has been effected, and the double-stranded region of the oligonucleotide including any sequence necessary to return the sequences that remain after cleavage into proper and original reading frame for expression; and

30 (v) cleaving the nucleic acid solely at a restriction endonuclease cleavage site contained within the double-stranded region of the partially double-stranded oligonucleotide, the site being different from that used in step (iii) or amplifying the nucleic acid

the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large enough region to allow the two strands to associate such that cleavage may occur at the chosen temperature and at the desired location, and the restriction being carried out using a cleavage endonuclease that is active at the chosen temperature; and

105. A library of immunoglobulins comprising members having at least one variable domain in which one or both of the CDR 1 and CDR 2 have synthetic diversity and the CDR 3 has diversity captured from B-Cells.

106. The library according to claim 104,
wherein a first variable domain has synthetic diversity
30 in CDR 1 and CDR 2 and has diversity in CDR 3 captured
from B-cells and a second variable domain has diversity
captured from B-cells.

107. The library according to claim 104 or 105, wherein the variable domain is selected from the group of VH or VL.

108. A method for cleaving a nucleic acid
5 sequence at a desired location, the method comprising the steps of:

10 (i) contacting a single-stranded nucleic acid sequence with a partially double-stranded oligonucleotide, the single-stranded region of the oligonucleotide being functionally complementary to the 5' terminal region of the nucleic acid sequence, the double-stranded region of the oligonucleotide including any sequences necessary to return
15 the sequence in the single-stranded nucleic acid sequence into proper and original reading frame for expression; and

20 (ii) cleaving the partially double-stranded oligonucleotide-single-stranded nucleic acid combination solely at a restriction endonuclease cleavage site contained within the double-stranded oligonucleotide or amplifying the combination using a primer at least in part functionally
25 complementary to at least part of the double-stranded region of the oligonucleotide, the primer introducing during amplification an endonuclease cleavage site and cleaving the amplified sequence solely at the site.

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109. The method according to claim 108,
wherein the length of the single-stranded portion of
the partially double-stranded oligonucleotide is
between 2 and 15 bases.

5 110. The method according to claim 109,
wherein the length of the single-stranded portion of
the partially double-stranded oligonucleotide is
between 7 and 10 bases.

10 111. The method according to claim 108,
wherein the length of the double-stranded portion of
the partially double-stranded oligonucleotide is
between 12 and 100 base pairs.

15 112. The method according to claim 111,
wherein the length of the double-stranded portion of
the partially double-stranded oligonucleotide is
between 20 and 100 base pairs.

20 113. The methods according to any one of
claims 99 to 104 and 108, further comprising at least
one nucleic acid amplification step between one or more
of steps (i) and (ii), steps (ii) and (iii), steps
(iii) and (iv) and steps (iv) and (v).

25 114. A library comprising a collection of
genetic packages that display a member of a diverse
family of peptides, polypeptides or proteins and
collectively display at least a portion of the
diversity of the family, the library being produced
using the methods of claims 99, 100, 103 or 113.

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115. A library comprising a collection of members of a diverse family of peptides, polypeptides or proteins and collectively comprise at least a portion of the diversity of the family, the library
5 being produced using the methods of claims 101, 102, 104 or 113.

116. The methods and libraries according to any one of claims 99 to 104 or 113, wherein the members of the library encode immunoglobulins.

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